RESEARCH PLAN PROPOSAL

Effect of Propachlor on the male reproductive toxicity in *Swiss albino* mice and role of pumpkin seed extract

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TABLE OF CONTENT

S.No	Title	Page No.
1.	INTRODUCTION	3-10
2.	REVIEW OF LITERATURE	11-14
3.	HYPOTHESIS	15
4.	OBJECTIVES	15
5.	PLAN OF WORK	16-19
6.	SIGNIFICANCE	19
7.	BIBLIOGRAPHY	20-22

INTRODUCTION

Herbicides, also commonly known as weed killers are chemical substances used to control unwanted plants. Selective herbicides control specific weed species, while leaving the desired crop relatively unharmed, while non-selective herbicides can be used to clear waste ground, industrial and construction sites, railways and railway embankments as they kill all plant material with which they come into contact. Apart from selective/non-selective, other important distinctions include *persistence* (also known as residual action), *means of uptake* and *mechanism of action*. Historically, products such as common salt and other metal salts were used as herbicides, however these have gradually fallen out of favor and in some countries a number of these are banned due to their persistence in soil, and toxicity and groundwater contamination concerns. Herbicides have also been used in warfare and conflict. Modern herbicides are often synthetic mimics of natural plant hormones which interfere with growth of the target plants.

Characteristics of Herbicides :Foliage-absorbed: herbicide kills all foliage contacted. Root-absorbed: herbicide is applied to the soil. Contact: herbicide must be applied directly to weeds, little or no movement to underground or shaded part of weed. Translocated: herbicide is absorbed and moves throughout the living portion of the plant (weed). Selective: differences between plant and weed (foliar characteristics, deactivation) makes herbicide selective. Non-Selective: herbicide kills most plants, must be applied in absence of desirable plants. Persistent: herbicide remains for weeks or months. Nonpersistent: herbicide remains for hours or days. Pre-Plant incorporated (PPI): herbicide application made prior to planting and mixed into the soil. Pre-Emergence: herbicide is applied after planting, but prior to crop and, generally, weed emergence. Post-Emergence: herbicide application occurs after crop emergence (also can refer to after weed emergence)

(www.arabis.org/images/.../WeedWeedControlNHerbicides/Herbicides)

APPLICATION

- Most herbicides are applied as water-based sprays using ground equipment.
- On large areas, herbicides may also at times be applied aerially using helicopters or airplanes, or through irrigation systems (known as chemigation).
- A further method of herbicide application developed around 2010, involves ridding the soil of its active weed seed bank rather than just killing the weed.

ADVANTAGES OF USING HERBICIDES

- Broad spectrum of weeds controlled
- Reduced crop injury
- Reduced herbicide carryover
- New mode of action for resistance management
- Crop management flexibility and simplicity

DISADVANTAGES OF HERBICIDES

- Mammalian toxicity
- Eco toxicity
- Weeds become super weeds
- Reduced crop yield
- Creates soil and air pollution

HEALTH AND ENVIRONMENTAL EFFECTS

- Herbicides have widely variable toxicity in addition to acute toxicity arising from ingestion of a significant quantity rapidly, and chronic toxicity arising from environmental and occupational exposure over long periods.

- Some herbicides cause a range of health effects ranging from skin rashes to death. The pathway of attack can arise from intentional or unintentional direct consumption, improper application resulting in the herbicide coming into direct contact with people or wildlife, inhalation of aerial sprays, or food consumption prior to the labelled preharvest interval.

- Under some conditions, certain herbicides can be transported via leaching or surface runoff to contaminate groundwater or distant surface water sources. Generally, the conditions that promote herbicide transport include intense storm events (particularly shortly after application) and soils with limited capacity to adsorb or retain the herbicides.

- Phenoxy herbicides are often contaminated with dioxins such as TCDD; research has suggested such contamination results in a small rise in cancer risk after occupational exposure to these herbicides.Triazine exposure has been implicated in a likely relationship to increased risk of breast cancer, although a causal relationship remains unclear.

(https://en.wikipedia.org/wiki/Herbicide)

EXAMPLE OF HERBICIDES

1) Aminopyralid is a broadleaf herbicide in the pyridine group, used to control weeds on grassland, such as docks, thistles and nettles.
2) Clopyralid is a broadleaf herbicide in the pyridine group, used mainly in turf,0 0r000angeland, and for control of noxious thistles.
3) Dicamba, a postemergent broadleaf herbicide with some soil activity, is used on turf and field corn.
4) Glufosinate ammonium, a broad-spectrum contact herbicide, is used to control weeds after the crop emerges or for total vegetation control on land not used for cultivation.
5) Fluroxypyr, a systemic, selective herbicide, is used for the control of broad-leaved weeds in small grain cereals, maize, pastures, rangeland and turf.
6) Glyphosate, a systemic nonselective herbicide, is used in no-till burndown and for weed control in crops genetically modified to resist its effects.
7) Imazamox, an imidazolinone manufactured by BASF for postemergence application that is an acetolactate synthase (ALS) inhibitor.
8) Paraquat is a nonselective contact herbicide used for no-till burndown and in aerial destruction of marijuana and coca plantings..
9) **Propachlor**, the preparation acts on annual grasses and on some broadleaf plants

(https://pubchem.ncbi.nlm.nih.gov/compound/propachlor)

PROPACHLOR

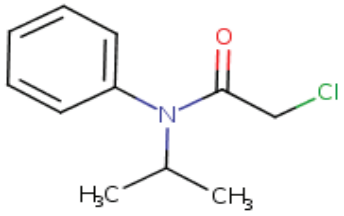
- Propachlor (2-chloro-*N*-isopropylacetanilide) an herbicide first marketed by Monsanto. It was registered for use in the United States during 1965.
- Propachlor can be applied with groundboom sprayers, tractor drawn granular broadcast spreaders, and granular row planters.
- Propachlor can be applied to the following crops/ areas: field corn, hybrid seed corn, silage corn, grain sorghum (milo), and onions grown for seed.

  (https://en.wikipedia.org/wiki/Propachlor)

Physical Description

PROPACHLOR is a light tan solid. Corrosive to iron and steel. Used as an herbicide.

Color/Form



Light tan solid

USES

- Propachlor, 2-chloro-N-isopropylacetanilide, is a selective herbicide used in commercial settings for the preemergence weed control of annual grasses and broadleaf weeds in grain sorghum (milo), field corn, hybrid seed corn, and silage corn, and for weed control.
- Herbicide for grasses & broadleaf weeds on corn, sorghum, soybeans, sugar beets and other field crops, forage crops, pasture land, & range land, summer fallow, & on vegetables.
- Selective pre-emergence, pre-planting, soil incorporated or early post-emergence herbicide to control annual grasses and some broad-leaved weeds in beans, brassicas, cotton, sorghum, peanuts, leeks, maize, onions, peas, roses, ornamental trees and shrubs, soya beans and sugar cane, at 3.36-6.72 kg/ha.
- Selective herbicide for control of annual grasses and some annual broad-leaved weeds in garlic, fennel, flower crops, flax, oilseed rape, strawberries, pumpkins.

HEALTH HAZARD

- Toxic inhalation, ingestion or skin contact with material may cause severe injury or death. Contact with molten substance may cause severe burns to skin and eyes

- Propachlor causes severe irritation of both eyes and skin in rodents

TOXICITY

- Propachlor can be absorbed into mammals through the respiratory and gastrointestinal tract as well as through the skin.
- Propachlor caused severe irritation effects on eyes and skin.
- When tested as a single dose in rats and mice, propachlor showed positive evidence of embryotoxicity. Embryotoxic effects were also observed in repeated dose regimens.
- In studies on soil microorganisms, nitrifying bacteria were the most sensitive group to the inhibitory effects of propachlor
- Propachlor is considered to be moderately to highly toxic to aquatic organisms.
- Propachlor is more toxic to birds when administered via the stomach than when fed in the diet. Propachlor does not pose a hazard to birds in the field, even with the granular formulation.
- Ingestions of these herbicides are likely to be followed by vomiting and diarrhea due to their irritant properties. (https://pubchem.ncbi.nlm.nih.gov/compound/propachlor#section=Information-Sources)

PUMPKIN (*Cucurbita maxima*) SEEDS

- Pumpkin belongs to the family of Cucurbitaceae. The pumpkin plant along with its seeds has been used in the traditional medicine in many countries, including India.

- Pumpkin contains calcium, iron, vitamin A, oil (25-55%, rich in unsaturated oleic and linoleic acids), protein (25-35%) with high amounts of arginine, aspartate and glutamic acid, although it is deficient in lysine and sulphur containing amino acids. The chemical constituents from seeds contain 30% unsaturated fatty oil (linoleic and oleic fatty acids). Triterpenoids, flavonoids, coumarins, saponines, cucurbitacins, antioxidant vitamins such as tocopherol and carotenoids, minerals, trace elements such as zinc and selenium, amino acid are also found in Pumpkin seeds. The highly unsaturated fatty acid composition of pumpkin seed makes it well-suited for improving nutritional benefits from foods

- Pumpkin seed extract is useful for immunomodulation, reproductive health and therapeutic advantage over a wide range of disease conditions. The antioxidative properties of pumpkin seed extract could also improve fertility and it helps to prevent arteriosclerosis, high blood pressure and heart diseases. (Aghaie *et al*, 2016).

- Pumpkin seeds improve libido and semen quality by elevating the serum testosterone level .It has been demonstrated that pumpkin seeds and daily rich diet of zinc can decrease the undesirable side effect of lead contaminants and improve the sexual health status . Also, pumpkin seed can inhibit citral induced hyperplasia of the ventral prostate lobe by reducing protein binding prostate levels (PBP) , weight of ventral prostate lobe and improve histology of testis. Therefore, it may be helpful in the management of benign prostatic hyperplasia. Pumpkin seeds improve sexual stimulation and intromission and ejaculatory latency**. (**Mohammadi *et al*, 2013).

DEXAMETHASONE

- **Dexamethasone** is a type of corticosteroid medication. It is used in the treatment of many conditions, including rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, and along with antibiotics in tuberculosis. In adrenocortical insufficiency, it should be used together with a medication that has greater mineralocorticoid effects such as fludrocortisone. In preterm labor, it may be used to improve outcomes in the baby. It may be taken by mouth, as an injection into a muscle, or intravenously. The effects of dexamethasone are frequently seen within a day and last for about three days.

- The long-term use of dexamethasone may result in thrush, bone loss, cataracts, easy bruising, or muscle weakness. Dexamethasone has anti-inflammatory and immunosuppressant effects.

- Toxic effects of dexamethasone on mouse testicular germ cells and concluded that Dexamethasone induces histopathological alterations such as epithelial vacuolisation, atrophy and reduction in testicular spermatozoid, reduction in tubular diameter and epithelial height, poor spermatogenesis, apoptotic index of germ cells was increased.(Orazizadeh *et al* 2009)

REVIEW OF LITERATURE

Propachlor-Herbicide Toxicity on Reproduction

- Mirkova *et al* (1975) reported action of the herbicide Ramrod on the embryogenesis of white rats and studied increase in the total embryonal lethality, conditioned entirely by the considerable rise, as compared to controls, of the pre-implantation deaths of embryos. The embryotoxic action of the preparation was furthermore manifested with a decrease in body weight and in the craniocaudal fetal sizes. Upon giving 1/5 and 1/10 LD50 Ramrod, a slight teratogenic effect was recorded. Ramrod at dose 1/40 LD50 exerted no effect whatsoever on embryogenesis.

- Zlateva *et al* (1979) studied disturbances in the processes of division and differentiation of the spermatopoietic epithelium in chronic Ramrod poisoning and inhibited spermatopoesis at the phase of the formation of spermatids, caused disorganization of seminal epithelium and occurrence of irregular dividing forms. There was dystrophic-degenerative changes in sex tissue during longer intoxication as well as occurrence of multinuclear gigantic cells. Disturbance in the kynetics of the mitotic division of spermatogonium and blockade of meiosis at its early phases were found as well. The authors assumed that damages in spermatopoiesis were due to distrubed metabolism of the whole organism. An important role was given to the vascular changes, which induced secondary enhancement of the metabolic disturbances in the gonads.

Propachlor-Herbicide Hepatotoxicity & Renal toxicity

- Davison *et al* (1990) reported metabolism of the glutathione conjugate of propachlor by *in situ* perfused kidneys and livers of rats and studied that mercapturic acid conjugates were found to be the end-products of metabolism of the 14C-propachlor-GSH by rat kidneys and liver, indicating that all enzymes necessary for the catabolism of GSH conjugates to mercapturates were present in each of these tissues. Perfusion of 14C-propachlor-GSH at 34 mumol/h appeared to exceed the metabolic capacity of both liver and kidneys for this substrate.

- Davison *et al* (1992) reported comparative metabolism of the cysteine and homocysteine conjugates of propachlor by *in situ* perfused kidneys of rats and studied that about half of the perfused 14C-labelled material isolated from urine from kidneys perfused with homocysteinyl propachlor was unchanged homocysteinyl propachlor and about half was the corresponding mercapturic acid. However, only the corresponding mercapturic acid and the S-oxide of this mercapturic acid (31.4% and 1.7% of the dose) were found in urine from kidneys perfused with cysteinyl propachlor, indicating that rat kidneys more efficiently acetylated the natural substrate, the cysteine conjugate.

- Mousal et al (2014) reported effect of propachlor toxicity on liver mice enzymes activity and the effect was studied in mammalian system (mice) depended on evaluating the enzymatic activity of liver function test (LFTs) enzymes: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP). The results showed that LFTs enzy mes decreased after seven days of gulping mice. Conclusion, propachlor showed acute liver damage, reflected from LFTs activity when compared with both vitamin C and phosphate buffer solution as a control.

Pumpkin seeds as protective

- Eraslan *et al* (2013) reported studies on the antioxidant effects of pumpkin seeds on subacute aflatoxin poisoning in mice was done by. It was determined that aflatoxin exhibited adverse effects on most of the oxidative stress markers. The administration of pumpkin seed diminished aflatoxin – induced adverse effects. The group which has given both aflatoxin and pumpkin seed were observed to have drawn closer to the values of the control group.

- Mohammadi *et al* (2013) reported effects of Herbal medicine on male infertility and concluded that medicinal herbs are used to treat sperm disorders, dysfunctioning of the libido, sexual asthenia and erection.

- Aghaie *et al* (2013) reported protective effect of Pumpkin seed extract on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with Cyclophosphamide and concluded that Pumpkin seed extract increase the total antioxidant capacity (TAC) level, improve the histopathological changes such as vacuolisation, disorganisation and separation of epididymal epithelium.

- Ayaz *et al* (2015) observed that evaluation of the anthelmintic activity of pumpkin seeds (*Cucurbita maxima)* in mice naturally infected with *Aspiculuris tetraptera* and concluded that Pumpkin seed has high anthelmintic activity against nematodes as well as its continued use in traditional medicine for the treatment of helminthiasis.

- Chari *et al* (2018) reported an appraisal of pumpkin Seed Extract in 1, 2 - Dimethylhydrazine induced colon cancer in Wistar rats and concluded that there was difference in the aberrant crypt foci (ACF) number in all treatment groups compared to control and Dimethylhydrazine groups, decrease in the colon length/weight ratio, change in the colonic glutathione (GSH) and superoxide dismutase(SOD) levels when compared to control and Dimethylhydrazine control and the nitrite content was decreased in the treatment group .

Dexamethasone as Positive control

- Khorsandi *et al* (2008) reported dexamethasone effects on Fas Ligand expression in mouse testicular germ cells and concluded that there was increase in apoptotic indexes of germ cells and increase in FasL expression of testicular germ cells.

- Orazizadeh *et al* (2009) studied toxic effects of dexamethasone on mouse testicular germ cells and concluded that Dexamethasone induces histopathological alterations such as epithelial vacuolisation, atrophy and reduction in testicular spermatozoid, reduction in tubular diameter and epithelial height, poor spermatogenesis, apoptotic index of germ cells was increased.

- Khorsandi *et al* (2013) reported effect of curcumin on dexamethasone-induced testicular toxicity in mice and concluded that Bcl-2 expression was increased in the curcumin + dexamethasone group compared with dexamethasone-treated animals. Dexamethasone induced spermatogenesis defects including epithelial vacuolizations, sloughing of germ cells, reduction of seminiferous tubule diameter, reduction in the number of sperm heads and significant maturation arrest.

HYPOTHESIS

Ho: There is no significant effect of propachlor on reproductive toxicity in male *Swiss albino* mice and pumpkin seed extract did not show significant protective role

H1: There is a significant effect of propachlor on reproductive toxicity in male *Swiss albino* mice and pumpkin seed extract show significant protective role

OBJECTIVES

To study the possible effect of propachlor on male reproductive toxicity in *Swiss albino* mice and role of pumpkin seed extract with the following objectives-

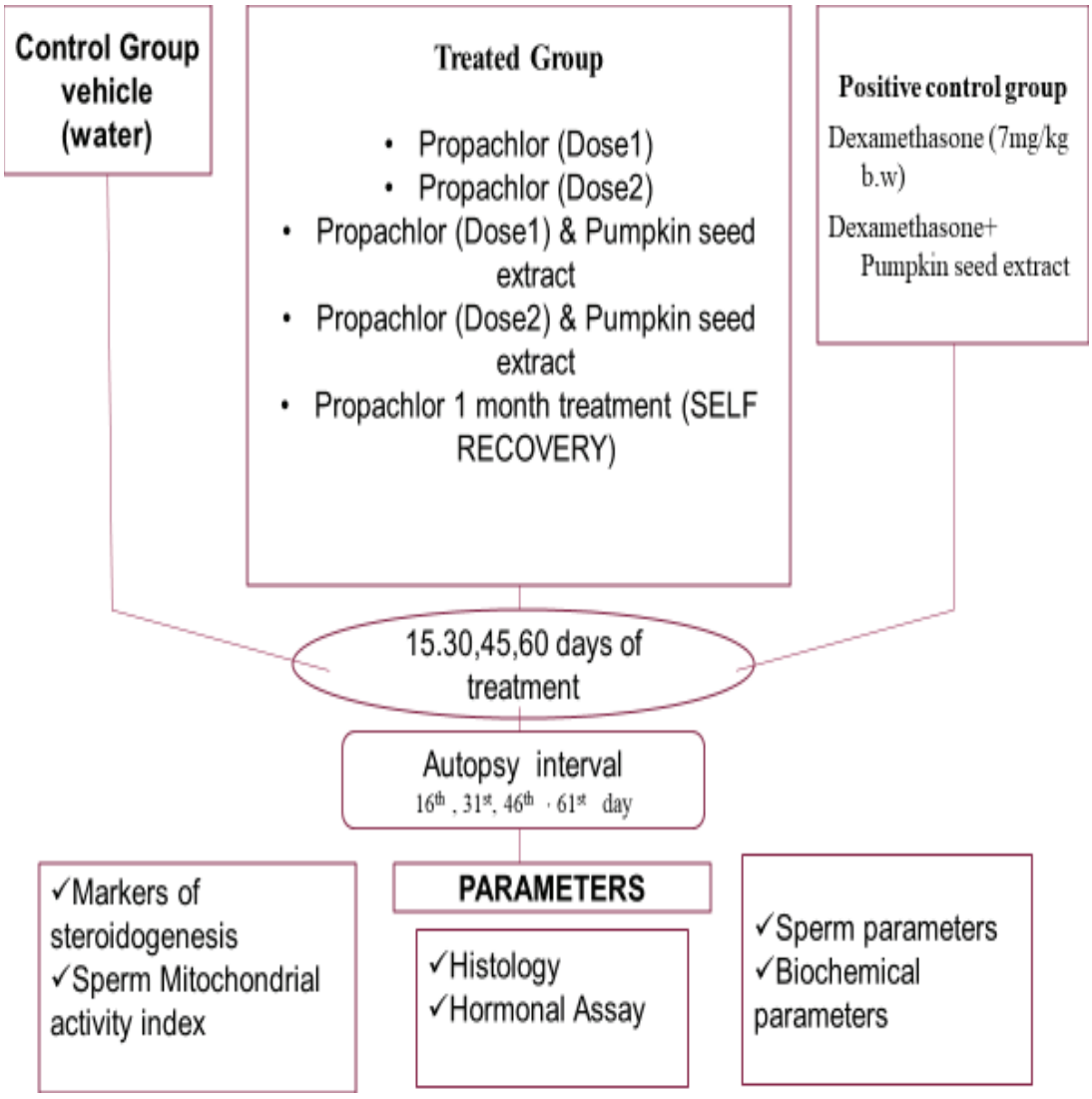
1. Observe the toxicity in reproductive parameters caused by continuous use of propachlor herbicide after 15, 30, 45 and 60 days of treatment.
2. Compare the propachlor induced changes with Dexamethasone (positive control) group on reproductive parameters.
3. Study the effect of Pumpkin seeds extract against propachlor on the reproductive parameters

PLAN OF WORK

Experimental Animal -

Animal to be used for this study shall be adult male *Swiss albino* mouse (*Mus musculus*), approximately 6-8 weeks old and weighing between 25 to 30g .

Experimental protocol- Mice will be divided into XII groups. In each group 12 animals will be used except in Group III, IV, VII, VIII, XII which will have 3 animals.



- Group I– Control Group (Vehicle water)
- Group II- Propachlor 145mg/kg ($1/50^{TH}$ LD_{50})
- Group III- Propachlor as in Group II for 30 days + Protective dose for next 15 days
- Group IV- Propachlor as in Group II for 30 days + Protective dose for next 30 days
- Group V- Simultaneously administration of Propachlor as in Group II + Protective dose for 15, 30,45, 60 days
- Group VI - Propachlor (Dose2)
- Group VII- Propachlor as in Group VI for 30 days + Protective dose for next 15 days
- Group VIII - Propachlor in Group VI for 30 days + Protective dose for next 30 days
- Group IX - Simultaneously administration of Propachlor as in Group VI + Protective dose for 15, 30,45, 60 days
- Group X- Dexamethasone positive control
- Group XI- Dexamethasone + Protective dose
- Group XII- Propachlor 1 month treatment (SELF RECOVERY)

Total number of animals required- 99 animals

- **Mode of Administration** – Intubation through gavage pipe.
- **Duration of Experiment**- 2 months
- **Duration of Treatment**- 15, 30, 45, 60 days
- **Autopsy** will be done at 16^{th} , 31^{st}, 46^{th} , 61^{st} day
- **Dose-**

Propachlor – 145mg/kg ($1/50^{TH}$ LD_{50}) and (Dose2)

Dose is selected from LD_{50} i.e 290mg/kg b.w (https://pubchem.ncbi.nlm.nih.gov/compound/propachlor#section=Information-Sources)

Pumpkin seed extract as protective

Dexamethasone as positive control - 7mg/kg body wt.

At the termination of experiment , animals will be sacrificed and the sample will be collected for further analysis or assessment.

Parameters will be done by following methods-

A. Physical parameters-

- Body weight
- Organ weight

B. Sperm Analysis-

- Sperm Motility (Prasad *et al*,1972)
- Sperm count (Prasad *et al*,1972)
- Sperm viability (Talpot and Chacon,1981)
- Sperm deformities (Bruce *et al*,1974)

C. Serum hormone level-

- Testosterone hormone
- Leutinizing hormone
- Follicle-stimulating hormone

D. Histology-

- Testis
- Epididymis

E. Tissue (Testis) Biochemistry -

- Protein (Lowry *et al*,1951)
- Cholestrol (Zlatkis *et al*,1953)
- Fructose (in Seminal vesicle) (Foreman *et a1* 1973)
- Sialic acid (Warren *et al*,1959)
- Glycogen (Seifter *et al*,1950)
- Ascorbic acid (Roe and Kuether, 1943)

F. Markers of Steroidogenesis-

- 3β Hydroxysteroid dehydrogenase (Talalay, 1962)
- 17β Hydroxysteroid dehydrogenase (Talalay, 1962)

G. Sperm Mitochondrial activity index-

Nitroblue Tetrazolium test

H. Statistical Analysis-

The results will be evaluated statistically by using SPSS software.

SIGNIFICANCE

- At what extent Propachlor cause changes in the reproductive parameters by its subchronic exposure
- To find the role of pumpkin seeds extract against propachlor

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